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Mapping Fusarium wilt race 1 resistance genes in cotton by inheritance, QTL and sequencing composition

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Abstract Knowledge of the inheritance of disease resistance and genomic regions housing resistance (R) genes is essential to prevent expanding pathogen threats such as Fusarium wilt [*Fusarium oxysporum* f.sp. *vasinfectum* (FOV) Atk. Sny & Hans] in cotton (*Gossypium* spp.). We conducted a comprehensive study combining conventional inheritance, genetic and quantitative trait loci (QTL) mapping, QTL marker-sequence composition, and genome sequencing to examine the distribution, structure and organization of disease R genes to race 1 of FOV in the cotton genome. Molecular markers were applied to F_2 and recombinant inbred line (RIL) interspecific mapping populations from the crosses Pima-S7 (*G. barbadense* L.) × 'Acala NemX' (*G. hirsutum* L.) and Upland TM-1 (*G. hirsutum*) × Pima 3-79 (*G. barbadense*), respectively. Three greenhouse tests and one field test were used to obtain sequential estimates of severity index (DSI) of leaves, and vascular stem and root staining (VRS). A single resistance gene model was observed for the F_2 population based on inheritance of phenotypes. However, additional inheritance analyses and QTL mapping indicated gene interactions and inheritance from nine cotton chromosomes, with major QTLs detected on five chromosomes [*Fov1-C06, Fov1-C08,* (*Fov1-C11*$_1$ and *Fov1-C11*$_2$), *Fov1-C16* and *Fov1-C19* loci], explaining 8–31% of the DSI or VRS variation. The *Fov1-C16* QTL locus identified in the F_2 and in the RIL populations had a significant role in conferring FOV race 1 resistance in different cotton backgrounds. Identified molecular markers may have important potential for breeding effective FOV race 1 resistance into elite cultivars by marker-assisted selection. Reconciliation between genetic and physical mapping of gene annotations from marker-DNA and new DNA sequences of BAC clones tagged with the resistance-associated QTLs revealed defenses genes induced upon pathogen infection and gene regions rich in disease-response elements, respectively. These offer candidate gene targets for Fusarium wilt resistance response in cotton and other host plants.

Keywords Cotton (*Gossypium*) · *G. barbadense* · *G. hirsutum* · Mapping · *Fusarium oxysporum* f.sp. *vasinfectum* · Disease resistance · Resistance gene cluster ·

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Quantitative trait loci · Transgressive segregation · Marker-assisted selection · Breeding · Genetics · Genomics · Quantitative disease resistance · R genes · PR-inducible defense-related genes

Introduction

The future of crop improvement depends on understanding the distribution, structure and organization of disease-resistance (R) genes. Diseases such as Fusarium wilt [*Fusarium oxysporum* f.sp. *vasinfectum* (FOV) Atk. Sny & Hans) in cotton represent expanding threats to crop production (Ulloa et al. 2010a; Kim et al. 2005). Increasing the knowledge of the inheritance of resistance to FOV and of genomic regions housing R genes will help to elucidate the mechanism of quantitative disease resistance. Genome sequencing, physical alignment of genomic regions into chromosomal maps and the anchoring of genetic maps are all steps that will improve the accuracy of detecting R genes underlying quantitative trait loci (QTL) and gene functions of important biological processes in crops (Chaudhary et al. 2008; Rong et al. 2004; Ulloa et al. 2007). In addition, molecular markers used on linkage maps and derived from cDNA (Arpat et al. 2004; Guo et al. 2007; Park et al. 2005; Ulloa et al. 2005) and genomic libraries (Frelichowski et al. 2006; Rong et al. 2004; Tomkins et al. 2001) have important implications for breeding effective pest and disease resistance into elite cultivars by marker-assisted selection (MAS). MAS reduces the time and labor spent on phenotype evaluations to identify resistant genotypes and speeds the breeding process.

Plants, unlike mammals, lack an immune system. Instead, they rely on the complex innate immunity of each cell and on somatic signals emanating from pathogen infection (PR) sites (Jones and Dangl 2006). The plant basal innate immune system can detect a wide range of microbe-associated molecular patterns and induce defenses (Bent and Mackey 2007). In general, plant disease resistance can be classified into two categories: qualitative resistance control by a single R gene and quantitative resistance control by more than one gene or QTL which each provides a partial increase in resistance. Disease-resistance genes can be divided further into two classes: (1) the host-pattern recognition receptor genes, which include the R genes that interact with pathogen-effector genes that suppress plant defenses and (2) inducible defense-responsive or defense-related genes that are activated upon PR (Bent and Mackey 2007; Kou and Wang 2010; van Loon et al. 2006). Plants express multiple R genes with specificities for different strains of viruses, bacteria, fungi, etc., and individual plant genomes include a few hundred R gene-like sequences (Bent and Mackey 2007; Adams-Phillips et al. 2008). Most R genes studied are members of a large gene family that all include leucine-rich repeat motifs (LRR) and nucleotide binding sites (NBS). Different R gene-mediated signal transduction pathways may utilize some distinct signaling components and induce a set of plant responses (Adams-Phillips et al. 2008; Sato et al. 2007). In contrast, PR genes have been classified into 17 families of pathogenesis-related proteins. These proteins are induced through the action of the signaling compounds of salicylic acid, jasmonic acid or ethylene. They exhibit antimicrobial activities in vitro through hydrolytic activities on cell walls, contact toxicity and perhaps an involvement in defense signaling. However, these proteins serve essential plant functions (senescence, wounding, cold stress and are present in floral tissue) whether they are induced in defense or not (van Loon et al. 2006).

Cotton (*Gossypium* spp.) is one of the most economically important crops, providing the world's leading natural fiber, and it is a model for cytogenetic, genomic and evolutionary biology research. Its fibers are thought to be the longest unicellular structures in the plant kingdom and are a model for research on cell elongation and cell wall biosynthesis (Chaudhary et al. 2008; Kim and Triplett 2001). Integrating disease-resistance phenotypes into high-yielding, high-fiber quality cultivars is one of the most important objectives in cotton breeding programs worldwide.

FOV is a soil-inhabiting fungus that can survive for long periods in the absence of a host. Consequently, it is nearly impossible to eradicate it from a field. Eight genotypes of FOV, called races, have been described throughout the world. Recently, highly virulent isolates of FOV were identified in cotton fields in Australia (Kochman et al. 2002; Wang et al. 2004) and California (Kim et al. 2005). In the USA, FOV race 1 causes yield losses in Acala, non-Acala Upland (*Gossypium hirsutum* L.) and Pima (*G. barbadense* L.) cottons. FOV race 1 is typically found in sandy or sandy-loam soils infested with root-knot nematode (*Meloidogyne incognita* [Kofoid and White] Chitwood) (Bell 1984; Veech 1984), which increases cotton susceptibility to FOV race 1 (Garber et al. 1979). Little is known about the genetic basis for cotton resistance to this and other FOV races, or how these races are affected by environmental factors and interactions with other pathogens (e.g., root-knot and reniform nematodes or Verticillium wilt).

Resistance to FOV has been examined in cotton under different phenotypic evaluation conditions and using different genetic backgrounds (Mohamed 1963; Smith and Dick 1960; Ulloa et al. 2006). Successive genetic analyses indicated that FOV resistance was determined by one or two major genes with complete to incomplete dominance, and possibly additional minor genes. Wang and Roberts

(2006) identified a major FOV race 1 resistance gene (*Fov1*) in *G. barbadense* cv. Pima-S7 based on inheritance in phenotypic studies and application of AFLP markers to segregating populations from the crosses Pima-S7 × Acala NemX and Pima-S7 × Acala SJ-2. A recent field study of FOV (Wang et al. 2009) revealed a major QTL on chromosome 17 contributing to race 7 FOV resistance in Upland cottons. Additional QTLs were reported from chromosomes 7, 15 and 23, suggesting a different specificity of resistance determinants to race 7 FOV and involvement of more than one gene.

Many phenotypic and mutant loci are assumed to have discrete phenotypes. However, some degree of phenotypic variation is often observed for many traits when they segregate in mapping populations with different genetic backgrounds. This variation suggests the interaction of two or more genes. For such traits, a QTL mapping approach can be informative for studying inheritance and detecting genomic regions and molecular markers tightly linked to the genes of interest (Ulloa et al. 2007, Ulloa et al. 2010c). Based on availability of genome-wide markers and mapping populations segregating for FOV resistance, we designed a study to investigate: (1) the inheritance of FOV race 1 resistance in two populations with different genetic backgrounds and levels of resistance; (2) the location and function of genes of the major cotton genome regions for FOV race 1 resistance using QTL; and (3) sequence compositions of candidate genes involved in FOV race 1 resistance.

Materials and methods

Fungus inoculum

An FOV isolate identified as race 1 (Kim et al. 2005; Wang and Roberts 2006) was used in greenhouse evaluations in 2006–2007 at the University of California, Riverside (UCR), CA and in a 2008 greenhouse evaluation at the University of California Kearney (UCK) Research and Extension Center (Parlier, CA, USA). Cultures from single spores were stored on filter paper at −20°C. To produce inoculum, the isolates were grown for 1–2 weeks at room temperature in 9-cm diameter Petri dishes containing 20 ml of potato dextrose agar (PDA) with 3 mM of streptomycin (Kim et al. 2005). Spore suspensions were prepared by flooding colonized 1–2-week-old cultures on PDA with water, scraping off the spores and filtering the spore suspension through four layers of cheesecloth (Ulloa et al. 2006, Ulloa et al. 2009). *Fusarium* spore suspensions were adjusted to the desired concentrations based on spore counts using a hemocytometer.

Plant material and Fusarium wilt race 1 assays

Plant genotypes used at the UCR site were susceptible *G. hirsutum* cvs. Acala SJ-2, Acala NemX and Upland TM-1, and resistant *G. barbadense* cvs. Pima-S7 and Pima 3-79. One interspecific cross was made [Pima-S7 × Acala NemX (Wang and Roberts 2006)], and the F_1 plants were selfed to produce an F_2 population. Parents, and F_1 and F_2 populations were assayed against race 1 of FOV. In addition, we used a recombinant inbred line (RIL) population developed from a cross between Upland TM-1 and Pima 3-79 (Kohel et al. 2001). This RIL population was used previously for mapping microsatellite or simple sequence repeat (SSR) markers (Frelichowski et al. 2006; Park et al. 2005). In the fall of 2005, a single plant from each RIL was transplanted into a pot in the greenhouse and the resulting seed was harvested from each plant in 2006. In 2007, the seeds from each RIL plant were planted in the field at Shafter, CA for seed increase, and 152 RILs produced seeds. Seeds of the RILs from these different generations were used in different FOV race 1 evaluations. In the UCR experiments, we used 139 (2006) or 138 (2007) RILs for assaying race 1 FOV infection in addition to the previously mentioned *G. hirsutum* and *G. barbadense* cultivars. The number of RILs evaluated depended on seed availability or germination.

A modification of a root-cut dip method described by Smith et al. (1981) was used in FOV race 1 greenhouse evaluations conducted in 2006–2007 at UCR. The roots of 1-week-old seedlings were rinsed with tap water, trimmed (secondary roots) to 2–3 cm length and immediately dipped for 3 min into an aqueous suspension of Fusarium macro- and micro-conidia (1×10^6 conidia/ml). Seedlings were then transplanted into 750 g of steam-sterilized U.C. Mix #2 soil (Baker 1957) in 878-ml plastic pots with five plants in each pot. Five pots of five plants for each of the parents and Acala SJ-2 were randomly positioned between the randomized RIL lines on a greenhouse bench to confirm uniformity of test conditions. Individual plants were rated for disease severity index (DSI) based on the following scale: 0 = no symptoms; 1 = epinasty and slight dwarfing; 2 = 1–30% of chlorotic leaves; 3 = 31–80% of chlorotic leaves and severe stunting; 4 = 81–100% of chlorotic leaves; and 5 = plant death. For plants with a DSI of ≥1, the stems and upper part of the primary root were cut longitudinally and evaluated for vascular root staining (VRS) at the end of the experiment. The following scale was used for VRS: 0 = no VRS, 1 = light VRS as spotty areas, 2 = more continuous than 1, but light-colored staining covering an area between one-quarter and one-half of the stem cross section, 3 = moderate brown/black staining evident in a band encircling most of the stem cross section, 4 = brown/black staining evident across most

vascular tissue in stem cross section, and 5 = plant severely damaged or plant death with staining evident throughout a cross section of root tissue (Ulloa et al. 2006, Ulloa et al. 2009).

At the UCR site, disease progress was monitored on 5 plants per RIL and 25 plants per parent and Acala SJ-2 based on DSI recorded at 9, 14 and 23 days after inoculation (dai) in 2006 and at 12, 14, 16, 19 and 23 dai in 2007 (Table 1). Mean phenotype scores from 5 plants per RIL were used for genetic mapping.

In the greenhouse evaluation at the UCK in 2008, parents (Upland TM-1 and Pima 3-79) of the RIL population and 140 RILs were seeded into a composite medium of vermiculite and peat moss prior to inoculation. Roots of 3-week-old seedlings were gently washed to remove most of the soil medium. The roots were then dipped for 2 min in FOV race 1 spore suspension of 1×10^5 conidia per ml of water. Seedlings were then transplanted into 6 × 15 cm (~400-ml) box pots with one plant in each pot. Plants from 137 RILs were assayed at 24 dai for VRS to determine the response to FOV race 1 infection. The greenhouse experiment used a completely randomized design with four replications. Each replication of an entry was represented by one plant per pot.

In 2007, 120 RILs were planted in a sandy-loam soil field site infested with FOV race 1 and *M. incognita* at the Shafter Research and Extension Center, Shafter, CA. Plants from previous studies in this field consistently developed severe FOV symptoms. Entries were grown in one-row plots, 5-m long with a 1-m row spacing in a randomized complete block design with two replications. Five plants randomly chosen per plot were used as subsamples to obtain estimates of VRS at 67 days after planting. In addition, plant survival in each plot was recorded at day 67 and again at 147 days after planting (Table 1). The percentage of plant survival was calculated by dividing the total number of surviving plants on each sample date by the initial plant count made 12–14 days after planting, and multiplying by 100.

Table 1 Summary of phenotypic evaluation tests for Fusarium wilt (FOV race 1) based on mean disease severity index (DSI) of leaves, as well as vascular stem and root staining (VRS) and standard deviation of an interspecific Upland TM-1 (*Gossypium hirsutum*) × Pima 3-79 (*G. barbadense*) recombinant inbred line (RIL) population

	RIL no	Mean	SD
Greenhouse test			
2006 Day 9[a]	139	2.84	1.37
2006 Day 14	139	3.68	1.23
2006 Day 23	139	4.56	0.88
2007 Day 12[b]	138	1.24	0.99
2007 Day 14	138	2.68	1.14
2007 Day 16	138	3.48	1.00
2007 Day 19	138	3.89	0.95
2007 Day 23	138	4.24	0.90
2008 Day 24[c]	137	2.52	1.08
Field test[d]			
2007 Day 67 after planting	113	1.61	0.71
2007 Day 67 %Survival	113	85.74	13.37
2007 Day 147 %Survival	113	27.39	19.36

[a] DSI greenhouse evaluation conducted in 2006 at the University of California Riverside, CA

[b] DSI greenhouse evaluation conducted in 2007 at the University of California Riverside, CA

[c] VRS greenhouse evaluation conducted in 2008 at the University of California Kearney Research and Extension Center, Parlier, CA

[d] VRS field evaluation conducted in 2007 on a site infested with FOV race 1 and *M. incognita* at the USDA-ARS, WICS, Research Unit, Shafter, CA

Marker analysis

We used 950 SSR markers with wide genome coverage. SSRs averaged 6 cM between two linked markers on cotton chromosomes (Frelichowski et al. 2006; Park et al. 2005; Ulloa et al. 2008; Wang et al. 2006; CMD, http://www.cottonmarker.org). In addition, we used another 250 markers obtained from the CMD public database (http://www.cottonmarker.org) and 100 in-house (USDA-ARS, WICS, Shafter, CA, USA) markers in the various experiments.

Polymerase chain reaction (PCR) amplification of BNL, CIR, Gh, MUSB, MUCS, MUSS and NAU cotton molecular markers was performed on a total volume of 15 μL containing 2 μL of DNA template (concentration 10 ng), 0.1 μM each of forward and reverse primers, 1× PCR buffer, 3 mM of $MgCl_2$, 0.2 mM of dNTPs and 0.5 U of Gold Taq polymerase (Amplitaq, Applied Biosystems, Foster City, CA, USA) with cycling profile of 1 cycle of 10 min at 94°C (hot start); 10 cycles of 15 s at 94°C, 30 s at 60°C (step −0.5°C/cycle for cycles 2–10) and 1 min at 72°C; 35 cycles of 15 s at 94°C, 30 s at 55°C and 1 min at 72°C; final extension cycle of 6 min at 72°C. PCR products were separated for 4–5 h on a 3% superfine resolution (SFR™) agarose gel (Amresco, Solon, OH) containing 1× TBE at 90 volts and were visualized by Alphaimager software (v. 5.5, Alpha Innotech Corporation, San Leandro, CA, USA) after staining with ethidium bromide. Primer pairs were scored if they resulted in discrete PCR banding patterns (amplicons) denoting a molecular marker.

We assessed SSR markers located on different cotton chromosomes using bulked segregant analysis (BSA) and QTL analysis (see below). The BSA method included resistant [(R) Pima-S7 and Pima 3-79] and susceptible [(S) Acala NemX and Upland TM-1] parents, and R and S

bulked F_2 progeny and RILs. Assayed plants from UCR with a DSI ≤ 2, or from UCK with VRS ≤ 2, were classified as R. Assayed plants with a DSI > 2 or VRS > 2 were classified as S. Previously reported estimates of DSI at 19 dai from the $F_{2:3}$ (Pima S-7 $\times$ NemX) population (Wang and Roberts 2006) were used to classify and select R and S F_{2s} for DNA bulked samples. DSI values from experiments at different dai in 2006 and in 2007 at UCR, and VRS values from the experiment in 2008 at UCK were used to classify and select R and S RILs for DNA bulked samples. Bulked samples included ten selected R and ten selected S entries from the F_2 and RIL populations across all experiments.

Data analyses

Correlation analyses were performed to examine the similarity of responses of the RIL entries in the various studies as indicated at different days after inoculation or using different indices of response (DSI, VRS, plant survival). Results of the UCR studies (2006, 2007) were examined for correlations between the DSI observed at all combinations of RIL dai and study (year). Similarity in responses of the RIL entries among studies was assessed by correlation analyses of DSI corresponding to each date in the two UCR experiments with VRS recorded at 24 dai in the UCK experiment (2008). Finally, concurrence of the VRS index with percent plant survival at 67 and 147 days after planting in the Shafter field study was assessed by correlation analysis. All correlations were performed using PROC CORR of SAS (ver. 9.2, SAS Institute, Cary, NC, USA).

Segregation ratios of resistant (R, DSI ≤ 2 or VRS ≤ 2) and susceptible plants (S, DSI > 2 or VRS > 2) were evaluated for entries from F_2 and RIL populations in each study. The goodness of fit of the observed R:S ratio to the expected Mendelian ratio was assessed by Chi-square analysis (Weir 1996).

Genetic linkage analyses

The informative bands were scored as present (+) or absent (−) for a dominant marker (expected ratios 3:1 or 1:3 for an F_2 or 1:1 for an RIL population), and if alleles from both parents were present, then the marker was scored as co-dominant (expected genotypic ratios 1:2:1 for an F_2 or 1:1 for an RIL population). The JoinMap® version 3.0 (Van Ooijen and Voorrips 2001) computer program was used to test for Chi-square goodness-of fit for expected versus observed genotypic ratios, and to construct the linkage groups/chromosomes for the RIL population. We used existing DNA of the F_2 (Pima-S7 $\times$ Acala NemX) mapping population and AFLP genotyped data from the Wang and Roberts (2006) study to construct the linkage group/chromosome 16 of the F_2 population. Likelihood ratio statistic (or LOD) scores of 4–16 were examined for each population using the Kosambi map function and a maximum distance of 40 cM. LOD threshold scores >6.0 were used as a cutoff to determine linkage between any two markers.

Quantitative trait loci analyses

Single-marker analysis was conducted using a nonparametric mapping test [Kruskal–Wallis analysis (K*)] equivalent to a one-way analysis of variance (Van Ooijen 2004). QTL analyses were conducted on DSI and VRS phenotypic data using MapQTL 4.0 with interval mapping, the multiple-QTL model and restricted multiple-QTL model mapping procedures. Threshold values for LOD were determined empirically after 100 permutation tests for all traits (Churchill and Doerge 1994). The LOD threshold for a QTL was determined at ≥ 3.0. QTL analyses in the RIL (Upland TM-1 $\times$ Pima 3-79) population were performed on the greenhouse DSI phenotypic data recorded at 9, 14 and 23 dai in 2006 at UCR; DSI data recorded at 12, 14, 16, 19 and 23 dai in 2007 at UCR; and VRS data recorded at 24 dai in 2008 at UCK. QTL analysis was also done using DSI data recorded at 19 dai of $F_{2:3}$ (Pima S-7 $\times$ NemX) families from the Wang and Roberts (2006) study. In addition, QTL analysis was performed on phenotypic VRS data recorded at 67 days after planting and percent plant survival data recorded at 67 and 147 days after planting from the field site co-infested with FOV race 1 and RKN (Table 1).

Data mining and BAC sequencing

We obtained the SSR sequence information from the Cotton Marker Database (CMD: http://www.cottonmarker.org/). Sequences were then blasted through the National Center for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/). Sequences were compared against four databases: (a) nucleotide collection (nr/nt), (b) expressed sequence tags (EST), (c) non-redundant protein sequences (nr), and (d) Swissprot protein sequences (SwissProt). The top sequence hits found for each sequence in all four databases were then blasted through gene ontology (GO) (http://www.geneontology.org/). The top functional hits given by GO were collected along with their categorized gene products [biological process (BP), cellular component (CC) and molecular function (MF)].

A small-insert (3–5 kb) library was constructed from a bacterial artificial chromosome (BAC) clone, which harbored the MUSB0827 SSR marker. This BAC clone was obtained from a BAC library developed at the Clemson

University Genomics Institute (CUGI) from the cotton cultivar Acala Maxxa (Tomkins et al. 2001). Small-insert DNA fragments were generated by isolating BAC DNA as a maxi-prep from the BAC clone and subjecting the DNA to random fragmentation by hydroshearing (Digilab®, Digilab Inc., Holliston, MA, USA). Fragments between 3 and 5 kb were size-selected by gel electrophoresis, subjected to end-repair, cloned into the hi-copy plasmid-based cloning vector pBlueskriptIIKSII+ (Stratagene, Agilent Technologies, La Jolla, CA, USA) and then electroporated into *E. coli* DH10B host cells. Transformants were selected on lysogeny broth (LB) plates containing carbenicillan, X-Gal and IPTG. White recombinant colonies were picked robotically using the Genetix Q-bot (Genetix, Boston, MA, USA) and stored as individual clones in Genetix 96-well microtiter plates as glycerol stocks at −80°C. Sequencing was performed using the dye-terminator cycle sequencing kit v3.1 (Applied Biosystems, Foster City, CA, USA). Sequence data from the forward and reverse universal priming sites of the shotgun clones were accumulated on an ABI 3730xl DNA analyzer.

The BAC clone was sequenced to approximate 8× clone coverage (assuming 120 kb average insert size), assembled with PHRAP software (Ewing et al. 1998) and edited with Consed (Gordon et al. 1998). Sequence contigs were ordered and oriented by the bridging shotgun method and gaps were joined by the addition of Ns giving a single contiguous consensus sequence for analysis. Gene prediction and annotation were performed with the gene prediction programs Augustus (Stanke and Morgenstern 2005) and FGENESH (Softberry Mount Kisco, NY, USA, http://www.softberry.com). Local alignments were made with the cumulative *Gossypium* unigene set from http://www.plantgdb.org. The Augustus program was trained on the *Arabidopsis* gene set, which considers EST matches as additional support for gene identification. The FGENSH program was trained on the *Arabidopsis* and *Vitis* gene sets. All predicted genes and unigene matches were blasted through the NR database to identify previously established protein motifs and then were subjected to domain searches with InterProscan (European Bioinformatics Institute, Cambridge, UK). Output from all prediction software and EST alignments were converted to GIF format for visualization with the Gbrowse software (Stein et al. 2002).

Results

Phenotypic resistance evaluations

In the experiment at UCR in 2006, the DSI ratings of susceptible parent Acala NemX of the F_2 (Pima-S7 × Acala NemX) population and susceptible entry Acala SJ-2 were >2.5 at 9 days after inoculation (dai), and at the end of the experiment all inoculated plants of Acala NemX and SJ-2 were dead (DSI = 5). The DSI of resistant parent Pima-S7 averaged 1.2, indicating that Pima-S7 was resistant but not immune to FOV race 1. Similar results for DSI were observed for susceptible Acala NemX and resistant Pima-S7 parents in the experiment at UCR in 2007. Based on the frequency distribution of the RIL populations, FOV race 1-induced DSI tended to increase with days after inoculation in the RIL population in both tests at UCR. In Fig. 1, the frequency distribution of the RIL population for the experiment at UCR in 2007 is presented. In the experiment at UCK in 2008, similar results were also obtained based on observed VRS ratings of susceptible Upland TM-1 and resistant Pima 3-79 parents of the RIL (TM-1 × Pima 3-79) population where the VRS mean was 3.5 for Upland TM-1 and 1.5 for Pima 3-79 at 24 dai.

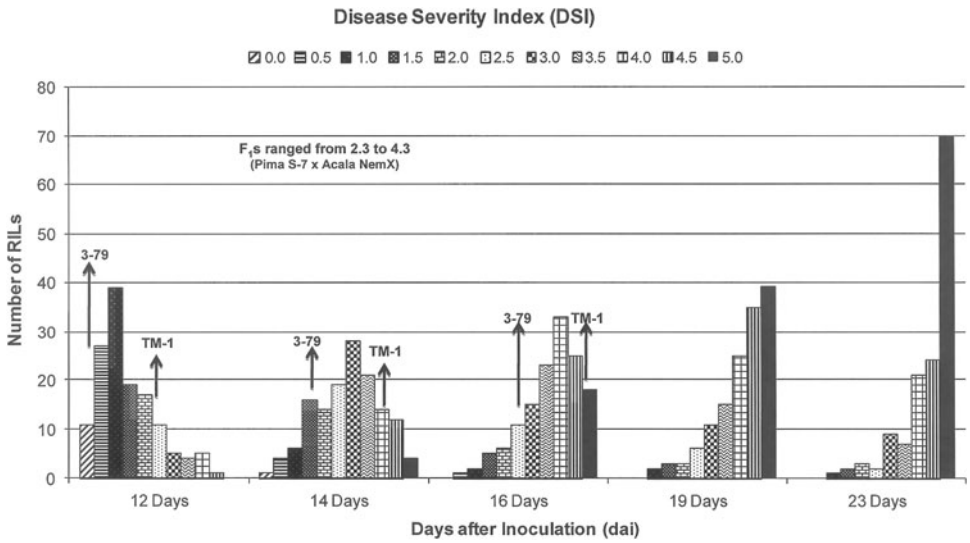

Fig. 1 Distribution of disease severity index (DSI) of leaves of Fusarium wilt (FOV race 1) on an interspecific [Upland TM-1 (*Gossypium hirsutum*) × Pima 3-79 (*G. barbadense*)] RIL population. DSI: 0–5 scale; 0 = no symptoms, and 5 = plant dead. *X* axis = number of individuals. *Y* axis = evaluation after days of inoculation (dai). DSI ranging from 0.4 to 3.0 for resistant Pima 3-79 and 2.3 to 5.0 for susceptible TM-1. Greenhouse evaluation conducted in 2007 at the University of California Riverside, CA

All correlations examining the relationships between the DSI of RIL entries observed at different dai in the 2006 and 2007 experiments at UCR, between the DSI observed in the UCR experiments and VRS observed at 24 dai at UCK, and between VRS and percent plant survival observed at Shafter were significant ($P < 0.05$). The Pearson correlation coefficients corresponding to comparisons within the UCR studies ranged from $r = 0.33$ (DSI at 23 dai in 2006 and 12 dai in 2007) to $r = 0.60$ (DSI at 14 dai in 2006 and 16 dai in 2007). Correlation coefficients corresponding to DSI observed at different dai in 2006 at UCR, and VRS observed at UCK, ranged from $r = 0.21$ (DSI at 23 dai) to $r = 0.27$ (DSI at 14 dai). Corresponding coefficients for relationships between DSI at UCR in 2007 and VRS at UCK ranged from $r = 0.22$ (DSI at 12 dai) to $r = 0.34$ (DSI at 14 dai). In the field test at Shafter, VRS ratings at 67 days after planting were negatively correlated with percent plant survival at both 67 days ($r = -0.65$) and 147 days after planting ($r = -0.68$).

Inheritance of resistance to Fusarium wilt (FOV) race 1

Wang and Roberts (2006) determined that a major gene (designated *Fov1*) conferred resistance to FOV race 1 in Pima-S7. Their F_2 segregation data from a Pima-S7 × Acala NemX population conformed to a single recessive gene model. However, in our examination of two segregating populations (F_2 Pima-S7 × Acala NemX and RIL Upland TM-1 × Pima 3-79), as the disease progressed and wilt severity increased, fewer resistant plants and RILs could be identified. The observed segregation was conditioned on the time of evaluation (days after inoculation, Fig. 1), suggesting that additional environmental and genetic factors were involved in FOV infection. Based on

Table 2 Observed and expected values and Chi-square values for a single and multiple gene models for response to *Fusarium oxysporum* f.sp. *vasinfectum* race 1 infection in an F_2 population from the cross of resistant Pima S-7 × susceptible Acala NemX, and a recombinant inbred line (RIL) population from the cross susceptible Upland TM-1 × resistant Pima 3-79 using root dip inoculation methods under greenhouse conditions

Genotype/population	Number of plants	Expected ratio R:S[a]	Observed ratio R:S	χ^2	*P* value
Parents					
Pima S7	43	All R	38:5		
NemX	44	All S	5:39		
F_1	66	All S	6:60		
F_2	133	33:100 (1:3)[d]	31:102	0.203	NS[‡]
Parents					
TM1	5	All S	1:4		
3-79	5	All R	5:0		
RIL (2006) Day 9[b]	139	35:104 (1:3)[e]	40:99	0.954	NS
		68:69 (1:1)[d]	40:99	6.25	**
Parents					
TM1	5	All S	0:5		
3-79	5	All R	4:1		
RIL (2007) Day 14[b]	138	35:103 (1:3)[e]	41:97	1.379	NS
		68:69 (1:1)[d]	41:97	8.02	**
Parents					
TM1	4	All S	1:3		
3-79	4	All R	4:0		
RIL (2008) Day 24[c]	137	34:103 (1:3)[e]	52:78	17.83	***
		68:69 (1:1)[d]	52:78	4.94	*

R resistant, *S* susceptible

‡ NS = not significant at $\chi^2_{0.05}$, * = significant at $> \chi^2_{0.05}$, ** = significant at $> \chi^2_{0.025}$, *** = significant at $> \chi^2_{0.005}$

[a] Expected number of plants for one and more than one gene models of resistance

[b] Disease severity index of leaves at 9 and 14 days after inoculation

[c] Vascular stem and root staining at 24 days after inoculation

[d] A single gene model

[e] A multiple gene model

DSI ratings from the 2006 and 2007 experiments at UCR (data not shown) and VRS ratings from the 2008 experiment at UCK on the Upland TM-1 × Pima 3-79 RIL population, we observed distorted Mendelian ratios for a single gene model. These ratios suggested that more than one gene was responsible for resistance to FOV race 1 in the progeny of this cross (Table 2).

All Upland TM-1 plants were highly susceptible to FOV race 1. However, plants with higher resistance than the resistant parents, Pima S-7 and Pima 3-79, were observed in some F_2 (Pima S-7 × NemX) families and RILs (Upland TM-1 × Pima 3-79), providing strong indications that multiple loci governed host-plant resistance to FOV race 1 (data not shown). The highly resistant families and RILs may carry two or more genes with one or more coming from each parent.

Molecular markers and genetic linkage analyses

Using BSA (Fig. 2), we screened 650 SSRs on R [DSI $\leq$ 2 or VRS $\leq$ 2 (Pima S-7 and Pima 3-79)] and S [DSI $>$ 2 or VRS $>$ 2 (Acala NemX and Upland TM-1)] parents, and R and S bulks of the F_2 progeny and RILs. We selected 50 potential R and S markers for race 1 FOV located on ten cotton chromosomes (data not shown). These SSRs showed different alleles or DNA fragment/amplicons associated with assayed R or S genotypes. A total of 24 SSRs, which amplified 33 amplicons or alleles, of these 50 markers are presented in Table 3.

As many as 1,050 SSR markers that provided wide genome coverage (Frelichowski et al. 2006; Park et al. 2005; Ulloa et al. 2008; Wang et al. 2006; CMD, http://www.cottonmarker.org) were tested on the RIL (Upland TM-1 × Pima 3-79) population for association with FOV race 1 resistance. Linkage analyses on the RILs used DNA isolated from FOV race 1-phenotyped plants (VRS) from the experiment in 2008 at UCK, together with a previously developed genetic linkage map (Frelichowski et al. 2006; Ulloa et al. 2008). Twenty-three linkage groups from the 24 cotton chromosomes were developed. We selected five linkage groups/chromosomes, which were identified by QTL analyses in this study. These five chromosomes were found to be involved in FOV race 1 resistance: chromosomes 6, 8, 11, 16 and 19 (Fig. 3). The linkage groups/chromosomes were developed with LOD $>$ 12 to obtain strong linkage between two anchored markers. We selected this high LOD score to represent specific chromosome regions of the cotton genome.

To develop chromosome 16 on the F_2 (Pima-S7 × Acala NemX) mapping population (Fig. 3), we used SSRs from the Upland TM-1 × Pima 3-79 RIL population of chromosome 16, genotypes based on two coupling-phase AFLP dominant markers (M-CAA_E-AAC-85 and M-CAA_E-AGC-169) and phenotypic data of the major recessive gene (*Fov1*), which determined resistance from Pima S-7.

Quantitative trait loci mapping for Fusarium wilt race 1 resistance

The nonparametric mapping test analogous to one-way analysis of variance (Van Ooijen 2004) revealed 24 SSR markers from nine chromosomes (1, 6, 8, 9, 11, 12, 15, 16 and 19) associated with FOV race 1 resistance. The 24 SSRs were identified using a developed genetic linkage map from FOV race 1-phenotyped plants based on VRS from the experiment in 2008 at UCK ($P < 0.05$) (Table 3; Fig. 3). These 24 markers yielding 33 alleles were again

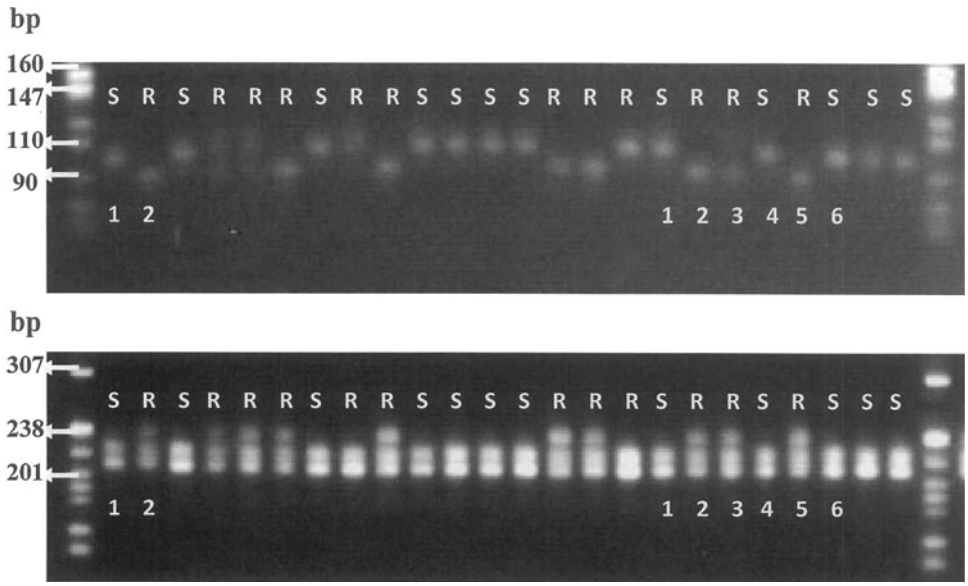

Fig. 2 Two microsatellites or SSR markers evaluated on susceptible (S) Upland TM-1 [No. 1, (*Gossypium hirsutum* L.)]; resistant (R) Pima 3-79 [No. 2, (*G. barbadense* L.)]; S and R RILs; R (No. 3) and S (No. 4) bulks of cotton RIL genotypes; Pima-S7 (R, No. 5); and Acala NemX (S, No. 6) for association with resistance to Fusarium wilt (FOV race 1)

Table 3 QTLs associated with disease severity index (DSI) of leaves, and vascular stem and root staining (VRS) of Fusarium wilt (FOV race 1) in an interspecific recombinant inbred line and F_2 mapping populations from two greenhouse tests and recorded DSI (2006) and VRS (2008) data detected by nonparametric mapping

Locus	Chromosome	TM-1: allele mean-a	Pima 3-79: allele mean-b	K*,†	*P* value‡
MUCS164_541 h[b]	1	2.84	2.18	10.59	***
Gh039_131/119[b]	6	3.04	2.20	12.29	***
BNL2569_164/169[b]	6	2.95	2.26	10.05	***
NAU2714_176/181[b]	6	3.20	2.31	17.20	***
MUSB0780_146/141[b]	8	2.76	2.29	5.26	**
NAU1037_208/221[b]	8	2.87	2.21	10.45	***
NAU0905_187b[b]	8	2.92	2.35	8.09	***
MUSS547_180b[b]	9	3.07	2.36	10.92	***
MUSB0850_248b[b]	11	2.92	2.17	11.72	***
NAU1014_276b[b]	11	3.00	2.41	7.42	***
MUCS399_219/224[b]	11	2.90	2.13	8.18	***
MUSB0155_120/129[b]	11	2.93	2.28	8.96	***
MUSB0155_136/127[a]	11	3.70	2.94	7.20	**
MUSB0117_142b[b]	12	2.93	2.38	8.15	***
MUSS128_245b[b]	15	2.93	2.27	8.58	***
NAU3901_305b[b]	15	2.91	1.77	22.51	***
BNL3008_144/155[b]	16	3.02	2.32	8.31	***
BNL3008_139/150[a]	16	4.17	3.10	8.23	**
Gh295_96/84[b]	16	2.92	2.33	7.25	***
Gh295_100/86[a]	16	4.17	3.10	8.82	**
MUCS594_240b[b]	16	3.07	2.27	15.48	***
MUCS594_269b[a]	16	4.42	3.60	5.32	**
Gh345_122/113[b]	16	2.97	2.14	15.07	***
Gh345_126/116[a]	16	4.52	3.12	11.83	***
MUCS616_237b[b]	16	3.23	1.97	37.20	***
MUCS616_256b[a]	16	4.56	3.57	5.80	**
MUSB0812_431 h[b]	16	3.42	2.00	42.86	***
MUSB0812_434/397[b]	16	3.37	1.93	46.76	***
MUSB0812_412/401[a]	16	4.52	2.92	13.14	***
MUSB0812_387 h[b]	16	3.34	1.94	45.40	***
Gh109_115b[b]	19	2.80	2.03	8.90	***
MUSS250_134 h[b]	19	2.88	2.26	9.56	***
NAU0980_206/218[b]	19	2.95	2.39	7.89	***

Analyses yielded 33 alleles from 24 simple sequence repeats or SSRs placed on nine cotton chromosomes

† Kruskal–Wallis analysis (K*) test regarded as the nonparametric equivalent of the one-way analysis of variance (Van Ooijen 2004)

‡ *P* values are designated as * $P < 0.1$,** $P < 0.05$ and *** $P < 0.01$

[a] QTL SSR markers associated with responses to FOV race 1 using recorded DSI on the Pima-S7 (b: allele) × Acala NemX (a: allele) F_2 mapping population from a greenhouse evaluation conducted in 2006 at the University of California Riverside, CA

[b] QTL SSR markers associated with responses to FOV race 1 using recorded VRS on the RIL Upland TM-1 × Pima 3-79, mapping population from a greenhouse evaluation conducted in 2008 at the University of California Kearney Research and Extension Center, Parlier, CA

[a, b] Alleles or DNA amplified fragments/amplicons differ because of agarose gel migration (variation $\pm$ 50 bp)

detected using a previously developed genetic linkage map (Ulloa et al. 2008) and phenotypes based on DSI (2006 and 2007 studies at UCR) or VRS (2008 study at UCK, data not shown).

Progeny homozygous for alleles identified by the SSR markers on the nine chromosomes from the resistant parents (Pima-S7 and Pima 3-79) were more resistant than those homozygous for the alleles from the susceptible

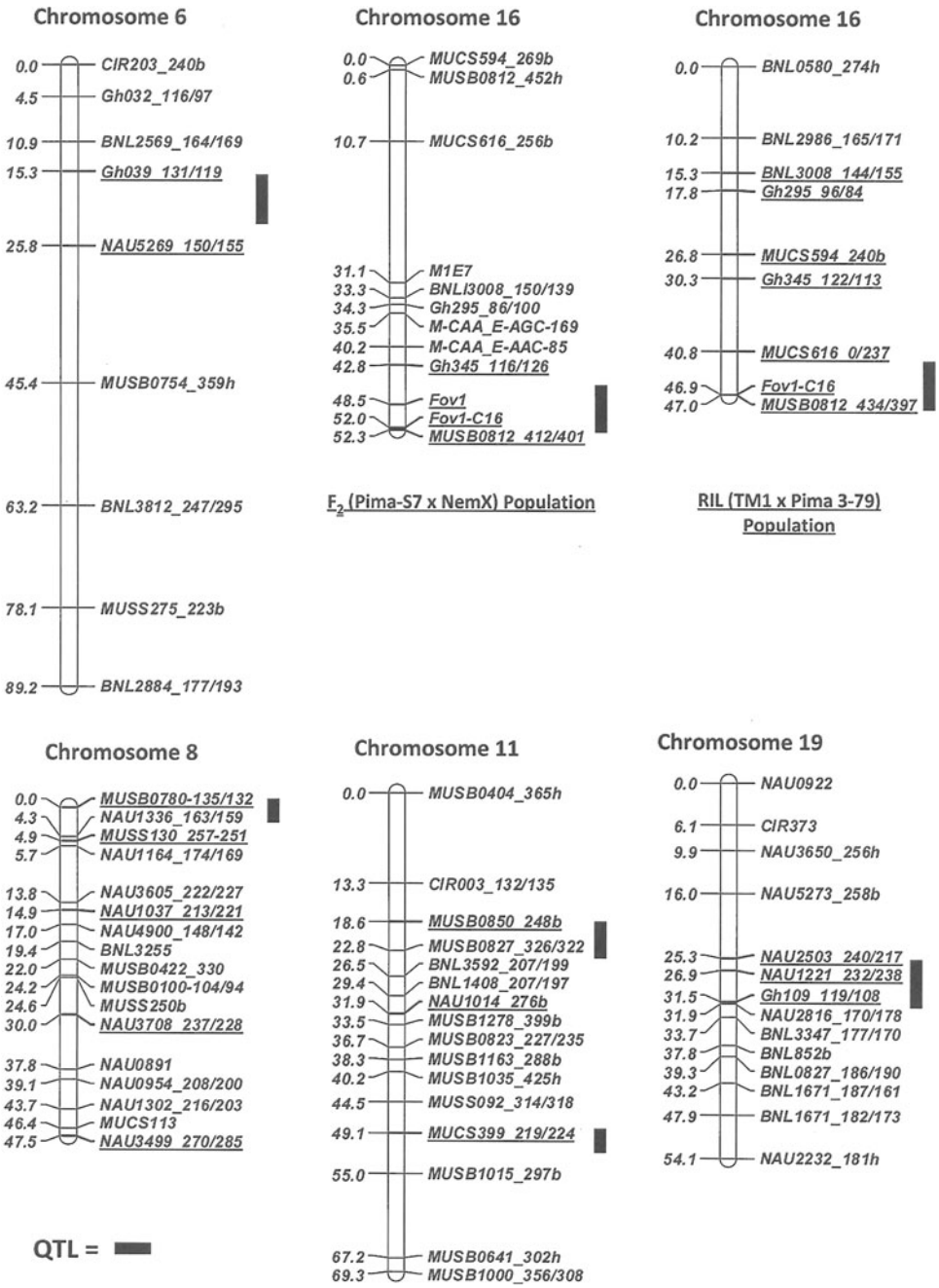

Fig. 3 Linkage maps of five chromosome regions (6, 8, 11, 16, and 19) showing the relationships between molecular markers and QTLs for Fusarium wilt (FOV race 1) resistance in an interspecific [Upland TM-1 (*Gossypium hirsutum*) × Pima 3-79 (*G. barbadense*)] RIL population

parents (Acala NemX and Upland TM-1, Table 3). One-way analysis of variance (Van Ooijen 2004) of DSI data recorded at 19 dai from an $F_{2:3}$ (Pima S-7 × NemX) population (Wang and Roberts 2006) also revealed seven alleles from two SSR markers, previously indentified on the RIL population, from two chromosomes (11 and 16) associated with FOV race 1 resistance ($P < 0.05$; Table 3). The observation of resistant progeny carrying alleles from the resistant parents is an indication of the additive effect of the contributing genes that would be subject to selection.

Common QTLs were detected on five linkage groups/chromosomes (chromosomes 6, 8, 11, 16, and 19) based on DSI and VRS data sets corresponding to the various experiments. There was general agreement among the data sets from greenhouse phenotyping (DSI from experiments in 2006 and 2007 at UCR, VRS from the experiment in 2008 at UCK) with regard to detection of QTLs inferred from statistical analyses. In the QTL analysis using VRS from the field experiment at 67 days after planting, we detected QTLs on chromosomes 8 and 19.

Major QTLs (Fig. 3) were detected and validated on five chromosomes by the multiple-QTL model and interval mapping analyses on the RIL population. On chromosome 6, we detected a QTL (LOD $\geq$ 3.0) between Gh039 and

NAU5269, explaining from 8.0 to 22.3% of variation in DSI, and having an additive effect ranging from 0.33 to 0.51. On chromosome 8, one QTL was detected near MUSB0780 marker (Fig. 3), explaining from 8.8 to 10.6% of DSI variation and with an additive effect ranging from 0.31 to 0.33. On chromosome 11, two QTLs (LOD $\geq$ 3.0), one near MUSB0850 and one near the MUCS399 marker, explained from 9.0 to 14.2% of DSI variation with an additive effect averaging 0.39 (MUSB0850) and −0.43 (MUCS399). On chromosome 16, a major QTL was detected with the highest observed LOD (>10) using VRS ratings from the study in 2008 at UCK (Table 3; Fig. 3). This QTL, associated with SSR marker MUSB0812, explained 31% of the variation in VRS with an additive effect of 0.72. On chromosome 19, a QTL was detected near SSR marker Gh109, explaining 13% of DSI variation with an additive effect of 0.46.

On chromosome 16 of the F_2 (Pima S-7 × NemX) population, a major QTL was associated with SSR marker MUSB0812 and explained 27% of DSI variation with an additive effect of 0.77. We used the DSI ratings at 19 dai for the $F_{2:3}$ (Pima S-7 × NemX) families to determine the F_2 phenotype. The *Fov1* major resistance locus from Pima-S7 was also mapped to chromosome 16 near marker MUSB0812. This genomic region also marked the QTL *Fov1*$_1$*-C16*, which made the biggest contribution to the FOV race 1 resistance phenotype contributed from Pima 3-79 to the RILs.

QTL analyses did not reveal strong QTLs (LOD > 3) associated with progression of wilt severity after inoculation or specific markers associated with early (DSI data at 9 dai in 2006 or at 12 dai in 2007 at UCR) or late (DSI data at 23 dai in 2006 or at 19 and 23 dai in 2007 at UCR) infection symptoms. Instead, we observed a set of markers from different chromosomes, which, as trait determinants, might interact to initiate the defense response to FOV race 1 infection.

Gene prediction from DNA sequences

Comparison of sequence information from 15 SSRs of the 24 markers on chromosomes 1, 6, 8, 9, 11, 12, 15, 16 and 19 (Table 4) to sequences in NCBI EST databases indicated considerable homology to genes for cotton fiber initiation (0–10 days post-anthesis) and elongation (7–10 day post-anthesis). In addition, the FOV race 1 resistance-associated marker Gh345 matched sequences known to be expressed in meristematic regions of roots (Table 4).

A total of 20 marker sequences had hits through gene ontology for genes from nine chromosomes (Table S1). The categorization of gene function revealed that markers mapped to genes associated with all three gene ontology categories of biological process, cellular component and molecular function (Table S1). Some of the BP assigned categories were associated with calcium ion transport, response to auxin stimulus/unidimensional cell growth, ethylene biosynthetic process/response to fungus, root development/shoot development, response to singlet oxygen/response to wounding and response to water deprivation/transport/water transport. For CC assigned categories, associations were found with: fungal-type vacuole, cell wall/vacuole, cytoplasm/chloroplast, cytosolic ribosome, chloroplast/plasma membrane and mitochondrion/chloroplast. MF assigned categories were mainly associated with calcium ion transmembrane transporter activity, indole-3-acetic acid amido synthetase activity, 1-aminocyclopropane-1-carboxylate oxidase activity and water channel activity among others (Table S1). Some of the above gene function categories may play a role in plant defense response or root development. In addition, a novel plant defense gene expressed in leaf tissue of *Arabidopsis* (ELI3) was identified for marker MUSB0850 (Table S1).

The 32 BAC clones tagged with BAC-end MUSB SSRs [selected from Frelichowski et al. (2006) and Ulloa et al. (2008)] were sequenced. These selected MUSB markers were identified as being associated with FOV resistance. The estimated BAC size according to assembled sequence data ranged from 68 to 140 kb with an average of 106 kb. Comparing initial blasted results against NCBI and the SwissProt databases indicated gene richness with considerable homology to disease-response elements for these BACs (complete analyses from sequences of all these BACs will be published separately). Herein, we report a single BAC clone that derived from the MUSB0827 SSR marker. BAC 33K23 was sequenced to an approximate 8× coverage, which resulted in three ordered contigs spanning 147,709 bp. The prediction software produced five potential genes (Table S2, Fig. 4). Gene regions of similarity to the retrotransposon *Ty3-gypsy* and polynucleotidyl ribonuclease h fold were predicted with up to 92% sequence homology to loci CAE75973.1 (*Oryza sativa* L.) and AAP4395.1 (*Gossypium herbaceum* L.). Gene ontology characterized these retroelements in two groups (biological process, cellular component) with implications in DNA metabolic process, nucleic acid binding, intracellular membrane-binding, catalytic activity and DNA integration.

Discussion

In this study, we conducted a comprehensive approach of combining conventional inheritance, genetic and QTL mapping, analysis of QTL marker-sequence composition and genome sequencing to examine the distribution,

Table 4 DNA sequence similarities of SSR markers associated with Fusarium wilt (FOV race 1) resistance obtained through the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/)

Locus	Chromosome	Tissue	Accession	Sequence description	Total score	Coverage (%)	*E* value	Max identity (%)
MUCS399	11	Fiber	ES808101.1	UFL_282_62 Cotton fiber 0–10 day post-anthesis (dpa) *Gossypium hirsutum* cDNA, mRNA sequence	1,439	92	0.00E+00	96
MUCS594	16	Fiber	ES843910.1	UFL_105_13 Cotton fiber 0–10 dpa *Gossypium hirsutum* cDNA, mRNA sequence	896	98	0	99
MUCS616	16	Fiber	ES815642.1	UFL_322_75 Cotton fiber 0–10 dpa *Gossypium hirsutum* cDNA, mRNA sequence	1,919	88	0	96
MUSS250	19	Fiber	ES810461.1	UFL_185_85 Cotton fiber 0–10 dpa *Gossypium hirsutum* cDNA, mRNA sequence.	1,729	93	0	95
NAU1037	8	Fiber	BQ404554.1	GA__Ed0072A06f *Gossypium arboreum* 7–10 dpa fiber library *Gossypium arboreum* cDNA clone GA__Ed0072A06f, mRNA sequence	1,264	100	0	100
NAU0905	8	Fiber	BQ411088.1	GA__Ed0036F04r *Gossypium arboreum* 7–10 dpa fiber library *Gossypium arboreum* cDNA clone GA__Ed0036F04r, mRNA sequence	1,146	100	0	100
MUSB0117	12	Fiber	BG441456.1	GA__Ea0013G22f *Gossypium arboreum* 7–10 dpa fiber library *Gossypium arboreum* cDNA clone GA__Ea0013G22f, mRNA sequence	1,260	49	0	99
NAU0980	19	Fiber	BQ407187.1	GA__Ed0103G07f *Gossypium arboreum* 7–10 dpa fiber library *Gossypium arboreum* cDNA clone GA__Ed0103G07f, mRNA sequence	1,099	100	0	100
NAU1014	11	Fiber	BQ405578.1	GA__Ed0084D08f *Gossypium arboreum* 7–10 dpa fiber library *Gossypium arboreum* cDNA clone GA__Ed0084D08f, mRNA sequence	1,195	100	0	100
MUSS128	15	Fiber	BG441456.1	GA__Ea0013G22f *Gossypium arboreum* 7–10 dpa fiber library *Gossypium arboreum* cDNA clone GA__Ea0013G22f, mRNA sequence	1,260	95	0.00E+00	99
MUCS164	1	Fiber	BG441791	GA__Ea0014K15f *Gossypium arboreum* 7–10 dpa fiber library *Gossypium arboreum* cDNA clone GA__Ea0014K15f, mRNA sequence	1,262	98	0.00E+00	100
MUSS547	9	Floral	CO118472.1	GR__Eb020N18.r GR__Eb *Gossypium raimondii* cDNA clone GR__Eb020N18 3′, mRNA sequence	1,264	98	0	96
MUSB0850	11	Leaf	XP_002322761.1	Cinnamyl alcohol dehydrogenase-like protein [*Populus trichocarpa*]	1,123	90	7.00E−57	99
Gh345	16	Meristematic region, very young fiber, roots, stem	DW485676.1	GH_RMIRS_054_B12_F Cotton Normalized Library random primed *Gossypium hirsutum* cDNA, mRNA sequence	148	18	3.00E−32	96
NAU2714	6	Whole seedlings	CO076382.1	GR__Ea37L04.r GR__Ea *Gossypium raimondii* cDNA clone GR__Ea37L04 3′, mRNA sequence	1,652	100	0	100

Sequences were blasted in expressed sequence tags (EST) database

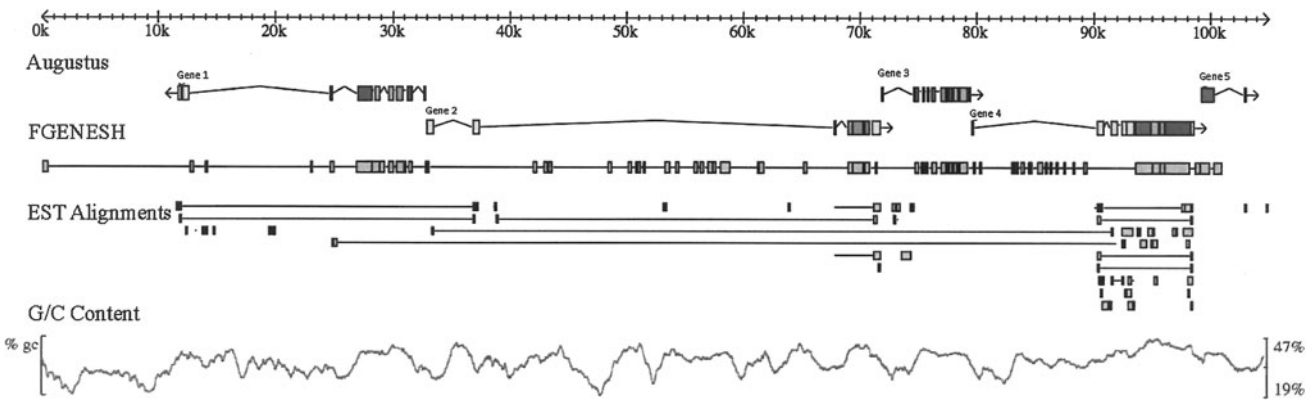

Fig. 4 Augustus and FGENESH (http://www.softberry.com) gene prediction and annotation and local alignments with the cumulative *Gossypium* unigene (EST) set from http://www.plantgdb.org of the Acala Maxxa BAC clone, which derived the SSR marker MUSB0827

structure and organization of disease-resistance (R) genes to Fusarium wilt in the cotton genome. Initially, a single resistance gene model was observed for the F_2 population based only on inheritance of phenotypes. The comprehensive genetic and QTL analyses yielded a set of 24 SSR markers and indicated the involvement of multiple genes and interactions for the inheritance of race 1 FOV resistance across nine cotton chromosomes (1, 6, 8, 9, 11, 12, 15, 16 and 19), with a major gene effect on chromosome 16. These markers have important application potential for breeding effective FOV race 1 resistance into elite cultivars by MAS. In addition, reconciliation between genetic and physical mapping of gene annotations from marker DNA and new DNA sequences of BAC clones tagged with the resistance-associated QTLs revealed defense genes induced upon PR and gene regions rich in disease-response elements, respectively. More research is needed to confirm which detected genes underlying QTLs have a function in race 1 FOV resistance. However, these results offer candidate gene sets for functional analyses of plant defense against Fusarium wilt and other biotrophic pathogens. This emerging knowledge will also help in developing the detected quantitative resistance genes with minor effects into a productive resource for crop improvement and protection.

The associations or correlations between the two disease evaluation indices suggest that DSI and VRS provide similar evidence of response to FOV infection by the RIL population. Relatively weak correlations between these indices (ranging from $r = 0.21$ to 0.34) observed at the UCR and UCK sites might be explained by between-site variation in the virulence of pathogen, differences in inoculum levels or differences in environmental conditions during the assays. Further optimization of phenotyping is needed to improve plant-resistance evaluations to minimize environmental variation and to increase resistance expression in segregating progeny.

In earlier studies under different evaluation conditions and using cultivars or progeny with different genetic backgrounds, FOV resistance was indicated to be under control of one or two major genes with complete to incomplete dominance, and possibly additional minor genes (Mohamed 1963; Smith and Dick 1960; Ulloa et al. 2006). Herein, we report a major gene (*Fov1*) with additive allele effects for resistance to FOV race 1 in Pima-S7 and Pima 3-79 (Table 2). The *Fov1* gene was previously identified by Wang and Roberts (2006) using progeny from crosses Pima-S7 × Acala NemX and Pima-S7 × Acala SJ2, and AFLP markers linked to this locus. However, as disease progressed and wilt severity increased over time from inoculation, especially for heterozygous progeny, fewer resistant plants and lines could be identified. The resulting Mendelian ratios were distorted for a single gene model and results suggested that more than one gene was responsible for resistance to FOV race 1 (Table 2; Fig. 3). The phenotypes of the progeny also revealed a transgressive effect with the susceptible parents Acala NemX or Upland TM-1 contributing one or more genes that enhanced resistance levels in progeny carrying gene *Fov1*.

Race specificity occurs in *F. oxysporum* f. sp. *vasinfectum*, a condition typically associated with major R gene-based qualitative resistance. Different genes also may be active in the plant disease response under different environmental conditions (Rebouillat et al. 2009). Greenhouse and field evaluations have revealed interactions in disease response between breeding lines, FOV races (1 and 4) and evaluation sites (greenhouse and infested field) (Ulloa et al. 2010a). We have observed that cultivars such as Pima S-7 and Phytogen 72 (an Acala Upland cotton) respond differently to two FOV races, with both cultivars being more susceptible to race 4 than to the complex of race 1 and root-knot nematode (Ulloa et al. 2010b). A recent field study of FOV (Wang et al. 2009) found a major QTL on chromosome 17 contributing to FOV race 7 resistance in Upland

cottons. Additional QTLs were reported from chromosomes 7, 15 and 23, suggesting a different specificity of resistance determinants to race 7 compared to our analysis of race 1 resistance. Collectively, these reports and the current results indicate that the race specificity for FOV resistance has a significant quantitative basis.

The polygenic basis of race 1 FOV resistance in the TM-1 × Pima 3-79 population was confirmed by the detection and validation of major QTLs on five cotton chromosomes (6, 8, 11, 16 and 19) using the multiple-QTL model and interval mapping analyses. We propose naming these QTLs following the conventional nomenclature where the acronym of the scientific name of the pathogen causing the disease is presented in lower case followed by the chromosome name. Moreover, the race number is added after the lower case of the name of the pathogen, and a number in subscript distinguishes multiple QTLs identified on the same chromosome. The following QTLs that explained from 8 to 31% of variation in DSI or VRS are designated here: *Fov1$_1$-C06* on chromosome 6 between markers Gh039 and NAU5269; *Fov1$_1$-C08* on chromosome 8, detected near marker MUSB0780; *Fov1$_1$-C11* near marker MUSB0850 and *Fov1$_2$-C11* near MUCS399, both on chromosome 11; *Fov1$_1$-C16* near marker MUSB0812 on chromosome 16; and *Fov1$_1$-C19* near marker Gh109 on chromosome 19 (Table 3; Fig. 3).

The genome region that marked the QTL *Fov1$_1$-C16* locus made the largest contribution (explaining 31% of the variation in VRS with an additive effect of 0.72) to the FOV race 1 resistance phenotype contributed by Pima 3-79 to the RILs. This QTL on chromosome 16, associated with SSR marker MUSB0812, was also detected in the F_2 Pima-S7 × Acala NemX population and explained 27% of variation in DSI with an additive effect of 0.77. Moreover, the major gene *Fov1* locus from Pima-S7 (Wang and Roberts 2006) was also mapped to chromosome 16 near marker MUSB0812. We conclude that this QTL has a significant role in conferring FOV race 1 resistance in different cotton backgrounds and should be a primary target trait for cotton breeding using MAS.

Our study provides greater insight into the organization and function of the cotton genome, as results indicated considerable homology of markers from chromosomes 1, 6, 8, 9, 11, 12, 15, 16 and 19 to genes for cotton fiber initiation (0–10 day post-anthesis) and elongation (7–10 day post-anthesis), and also genes expressed in meristematic regions and roots (Table 4). The categorization of gene function within all three gene ontology categories of biological process, cellular component and molecular function (Table S1) also revealed high homology to R genes or inducible defense-related genes and that these QTL marker genes may play a role in plant defense response or root development. Plant roots have a large range of functions, which include uptake and transport of water and nutrients as well as structural support. The identity and number of genes involved in cotton root initiation and development are poorly understood. Assuming similar mechanisms of cell growth and development, the genes identified for fiber initiation and elongation (Table 4) may also be expressed as a defense response to Fusarium wilt infection. Functional analysis of the candidate genes will be required to test this possibility.

DNA sequence information from genomic regions and markers, such as BACs and MUSB BAC-derived SSRs [M Ulloa, PA Roberts, C Saski, unpublished data; (Table S2, Fig. 4)], are valuable for cloning sequences, such as retroelements (Taliercio and Ulloa 2003). These retroelements may impact on genome structure and evaluation, and loci that effect plant–pathogen interactions (De Kock et al. 2005). Genetic and physical framework mapping in cotton are used to discover putative gene sequences involved in resistance to FOV, nematodes and possibly other common soil pathogens. For example, chromosome 11 houses resistance to race 1 FOV and root-knot nematode (Wang et al. 2006), reniform nematode (Dighe et al. 2009) and Verticillium wilt (Bolek et al. 2005). Additional genome sequencing coupled with physical alignment of genomic regions into chromosomal maps will expedite discovery of R or PR genes underlying QTLs and will offer candidate gene sets for functional analyses of plant defense against Fusarium wilt and other biotrophic pathogens. Our results provide excellent gene targets for this approach.

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